Journal of Biotechnology 184 (2014) 74–83

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Dual-mode cultivation of *Chlorella protothecoides* applying inter-reactors gas transfer improves microalgae biodiesel production

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ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 2 May 2014
Accepted 12 May 2014
Available online 23 May 2014

Keywords:
Chlorella protothecoides
Heterotrophic
Autotrophic
Biodiesel
Carbon dioxide fixation

ABSTRACT

Chlorella protothecoides, a lipid-producing microalga, was grown heterotrophically and autotrophically in separate reactors, the off-gases exiting the former being used to aerate the latter.

Autotrophic biomass productivity with the two-reactor association, 0.0249 g L^{-1} h^{-1}, was 2.2-fold the value obtained in a control autotrophic culture, aerated with ambient air. Fatty acid productivity was 1.7-fold the control value.

C. protothecoides heterotrophic biomass productivity was 0.229 g L^{-1} h^{-1}. This biomass' fatty acid content was 34.5% (w/w) with a profile suitable for biodiesel production, according to European Standards.

The carbon dioxide fixed by the autotrophic biomass was 45 mg CO_2 L^{-1} h^{-1} in the symbiotic arrangement, 2.1 times the control reactor value.

The avoided CO_2 atmospheric emission represented 30% of the CO_2 produced in the heterotrophic stage, while the released O_2 represented 49% of the oxygen demand in that stage.

Thus, an increased efficiency in the glucose carbon source use and a higher environmental sustainability were achieved in microalgal biodiesel production using the proposed assembly.

1. Introduction

Microalgae are a promising, alternative source of oil for biodiesel production, showing many advantages over the present sources, mainly higher plants. The main advantages offered by microalgae are higher photosynthetic efficiency and biomass productivity when compared to plants. Additionally, microalgae cultures can use non-arable land and non-potable water, do not compete with food, and their production is not seasonal, allowing daily harvesting. The oil yield from algae is estimated to be 9-fold higher than the highest oil yield in plants, reported for palm (Chisti, 2007).

However, microalgal biomass production is, at present, still more expensive than growing plants. The key factors affecting profitability in this industry are capital costs, directly affected by biomass productivity, together with the market price of the products, presently only high-value products (Stephens et al., 2010).

Therefore, further research is necessary before economic viability of biodiesel production from algae can be attained.

To improve microalgae productivity, the use of fully controlled closed bioreactors is a necessary tool. When compared to open reactors, closed photobioreactors generally provide higher biomass concentrations, higher growth rates and reduced risk of microbial contamination (Tredici, 2004). Moreover, heterotrophic growth in fermenters can reduce the production cost of microalgal biomass by a factor of ten (Lee, 2004). The microalga *Chlorella protothecoides* is an excellent candidate for biodiesel production, since it can achieve high biomass productivity and high lipid content values, with a suitable fatty acid profile for biodiesel production, when grown under selected conditions. Furthermore, it is able to grow both photoautotrophically and heterotrophically, thus offering the alternative of photobioreactors and fermenters for its growth.

Several studies have been reported using *C. protothecoides* for a variety of purposes. In earlier studies the *C. protothecoides* strain CS-41 from the CSIR Marine Laboratory, Australia (Shi et al., 1999), was heterotrophically cultured in glucose-limited batch fermentation (3.7 L), at 28 °C, pH 6.6, agitation speed 480 rpm and dissolved oxygen above 50% of saturation, to produce lutein-rich biomass. Using an initial glucose concentration of 40 g L^{-1} the culture attained a maximum biomass concentration of 18.4 g L^{-1} after 178 h, with a productivity of 0.103 g L^{-1} h^{-1}; after glucose depletion, this *Chlorella* strain went on to attain a maximum lutein content of 4.4 mg g^{-1} biomass dry weight. The specific growth rate of this strain was 0.0417 h^{-1} and the yields on consumed

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http://dx.doi.org/10.1016/j.jbiotec.2014.05.012
0168-1656/